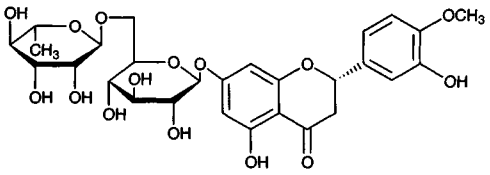
Hesperidin



Molecular formula: $C_{28}H_{34}O_{15}$
Molecular weight: 610.57
CAS Registry No.: 520-26-3
Merck Index: 4705

SAMPLE

Matrix: blood, urine
Sample preparation: Split each plasma and urine sample into two fractions. Mix 3 mL 1 M pH 4.5 sodium acetate buffer with one 3 mL plasma portion or one 10 mL urine portion, incubate at 37° for 20 h with 10,000 U β-glucuronidase (Helix Pomatia, Sigma). Condition a Sep-Pak C18 SPE cartridge with 6 mL EtOH:water 95:5 and 10 mL water. Add the sample to the SPE cartridge. Wash with 4 mL MeOH:water 10:90, elute the flavanones with 3 mL MeOH, reduce the plasma eluent volume to 500 μL under a stream of nitrogen. Inject 25 or 50 μL aliquots.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax ODS
Mobile phase: MeOH:water:glacial acetic acid 47:50.5:2.5
Flow rate: 1
Injection volume: 25-50
Detector: UV 280

CHROMATOGRAM

Retention time: 6.5
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES

Extracted: hesperitin, naringenin, naringin, narirutin

KEY WORDS

plasma; SPE

REFERENCE

Ameer,B.; Weintraub,R.A.; Johnson,J.V.; Yost,R.A.; Rouseff,R.L. Flavanone absorption after naringin, hesperidin, and citrus administration, *Clin.Pharmacol.Ther.*, **1996**, *60*, 34–40.

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a 2.4 mL 500 mg 40 μm Bond Elut C2 SPE cartridge with 2 mL MeOH and 2 mL water. Adjust pH of 1-2 mL urine or plasma to 5.5 with buffer, add to the SPE cartridge, wash with two 2 mL portions of water, elute with 1 mL MeOH. Add the eluate to 100 μL 4 M pH 5.5 ammonium acetate, centrifuge at 1000 g for 5 min, inject an aliquot of the supernatant. (Buffer was 10 mM ammonium acetate adjusted to pH 5.5 with acetic acid.)

HPLC VARIABLES

Column: 300 × 3.8 10 μm μBondapak C18
Mobile phase: Isopropanol:buffer 20:80 (Buffer was 10 mM ammonium acetate adjusted to pH 5.5 with acetic acid.)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 303

CHROMATOGRAM

Retention time: 4.69
Internal standard: hesperidin

OTHER SUBSTANCES
Extracted: flavone acetic acid

KEY WORDS
SPE; hesperidin is IS; plasma

REFERENCE
Cummings,J.; Kerr,D.J.; Kaye,S.B.; Smyth,J.F. Optimisation of a reversed-phase high-performance liquid chromatographic method for the determination of flavone acetic acid and its major human metabolites in plasma and urine, *J.Chromatogr.*, **1988**, *431*, 77–85.

SAMPLE
Matrix: fruit
Sample preparation: Grind 1.2 g dried fruit, extract with 50 mL EtOH:water 80:20 at 90° for 2 h. Filter the solution and evaporate it to dryness at vacuum. Dissolve 120 mg residue in 50 mL MeOH, filter (0.45 μm nylon Acrodisk), inject a 3 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.1 5μm Waters Symmetry C18 (Waters USA)
Mobile phase: Gradient. A was water containing 0.6% acetic acid. B was MeOH. A:B 80:20 to 60:40 over 12 min; maintain at 60:40 for 7 min, to 0:100 over 11 min, maintain at 0:100 for 3 min, to 80:20 over 2 min.
Column temperature: 45
Flow rate: 0.2
Injection volume: 3
Detector: UV 290; MS, HP 5989 B electrospray, quadrupole temperature 150°., EM 2173 V, positive mode, drying gas nitrogen, 360°., nebulizing gas nitrogen 0.55 MPa, m/z 611

CHROMATOGRAM
Retention time: 17.6

OTHER SUBSTANCES
Extracted: hesperitin, isonaringin, naringenin, naringin, neohesperidin, nobiletin, tangeritin

KEY WORDS
sour orange; orange

REFERENCE
He,X.-g.; Lian,L.-z.; Lin,L.-z.; Bernart,M.W. High-performance liquid chromatography--electrospray mass spectrometry in phytochemical analysis of sour orange (Citrus aurantium L.), *J.Chromatogr.A*, **1997**, *791*, 127–134.

SAMPLE
Matrix: juice
Sample preparation: Centrifuge 10 mL juice at 2500 g for 10 min, filter 1.2 μm, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 Spheri-5 C18
Column: 125 × 3.6 3 μm C18 (Supelco)
Mobile phase: MeCN:water:glacial acetic acid 20:79.5:0.5
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Extracted: narirutin, naringin, neohesperidin

KEY WORDS
orange; grapefruit

REFERENCE
Rouseff,R.L. Liquid chromatographic determination of naringin and neohesperidin as a detector of grapefruit juice in orange juice, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 798–802.

SAMPLE
Matrix: juice
Sample preparation: Condition a 360 mg Sep-Pak C18 SPE cartridge with 2 mL MeOH and two 4 mL portions of water. Centrifuge 15 mL orange juice at 8000 rpm for 15 min, add 2 mL of the supernatant to the SPE cartridge, wash with 2 mL water, wash with 1.5 mL MeOH: water 25:75, elute with 1.5 mL THF, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 mm long LC-NH2 (Supelco)
Column: 250 × 4.6 Bondesil C18 (Analytichem)
Mobile phase: Gradient. MeCN:THF:2% acetic acid 5:12:83 for 22 min, to 0:35:65 over 6 min, maintain at 0:35:65 for 12 min, return to initial conditions over 2 min, re-equilibrate for 10 min.
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Extracted: caffeic acid, coumaric acid, ferulic acid, narirurin, sinapic acid

KEY WORDS
orange; SPE

REFERENCE
Rouseff,R.L.; Dettweiler,G.R.; Swaine,R.M.; Naim,M.; Zehavi,U. Solid-phase extraction and HPLC determination of 4-vinyl guaiacol and its precursor, ferulic acid, in orange juice, *J.Chromatogr.Sci.*, **1992**, *30*, 383–387.

SAMPLE
Matrix: juice
Sample preparation: Grapefruit. 1 g Grapefruit juice concentrate + 1 mL 1.8 mg/mL rhoifolin in MeOH, vortex for 1 min, centrifuge at 25000 g for 15 min, remove the supernatant. Add 1 mL MeOH to the solid and stir with a spatula, vortex for 1 min, centrifuge, repeat extraction. Combine the supernatants and add 2 mL water, filter (1 μm glass fiber), filter (0.2 μm, Anotop), inject a 20 μL aliquot of the filtrate. Orange. 1 g Orange juice concentrate + 0.4 mL 1.8 mg/mL rhoifolin in MeOH + 3 mL MeOH, vortex for 30 s, heat at 55° for 15 min, mix for 30 s, centrifuge at 25000 g for 15 min, remove the supernatant. Add 2 mL MeOH and 1 mL water to the solid, vortex for 30 s, heat at 55° for 15 min, vortex for 30 s, centrifuge. Combine the supernatants and add 4 mL water, vortex, filter (1 μm glass fiber), filter (0.2 μm, Anotop), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: MeCN:water:isopropanol:formic acid 11.5:79:9.5:0.1
Flow rate: 0.6
Injection volume: 20
Detector: UV 283

CHROMATOGRAM
Retention time: 21
Internal standard: rhoifolin (UV 335) (30)

OTHER SUBSTANCES
Simultaneous: naringin, narirutin

KEY WORDS
concentrate; orange; grapefruit

REFERENCE
Bronner,W.E.; Beecher,G.R. Extraction and measurement of prominent flavonoids in orange and grapefruit juice concentrates, *J.Chromatogr.A*, **1995**, *705*, 247–256.

SAMPLE
Matrix: plants
Sample preparation: Grind wood, bark, or leaves and extract with MeOH:water 80:20 at room temperature for 24 h, filter, remove the MeOH under reduced pressure, extract the aqueous layer with diethyl ether. Dry the organic layer and evaporate it to dryness, reconstitute with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: Hypersil ODS
Column: 200 × Hypersil ODS
Mobile phase: Gradient. A was MeOH containing 0.1% phosphoric acid. B was water containing 0.1% phosphoric acid. A:B from 20:80 to 100:0 over 40 min, maintain at 100:0 for 5 min.
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Extracted: flavanoids, phenolic acids

KEY WORDS
wood; bark; leaves

REFERENCE
Conde,E.; Cadahía,E.; Garcia-Vallejo,M.C. HPLC analysis of flavonoids and phenolic acids and aldehydes in Eucalyptus spp, *Chromatographia*, **1995**, *41*, 657–660.

SAMPLE
Matrix: plants
Sample preparation: Extract leaves eight times with 5 mL MeOH at 60°, combine the extracts, filter, evaporate to dryness, reconstitute in 1 mL MeOH, filter (0.22 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18 (Vydac)
Mobile phase: Gradient. MeCN:3% acetic acid from 1:99 to 10:90 over 15 min, to 15:85 over 15 min, to 25:75 over 20 min, to 35:65 over 20 min, to 50:50 over 15 min, maintain at 50:50 for 20 min.
Column temperature: 28
Flow rate: 1
Detector: UV 255 or 280

CHROMATOGRAM
Retention time: 39.5
Internal standard: isorhamnetin 3-rutinoside (38)

OTHER SUBSTANCES
Simultaneous: caffeic acid, chlorogenic acid, daticoside, dihydrorobinetin, robinin, rutin

REFERENCE
Ficarra,R.; Ficarra,P.; Tommasini,S.; Calabrò,M.L.; Ragusa,S.; Barbera,R.; Rapisarda,A. Leaf extracts of some Cordia species: Analgesic and anti-inflammatory activities as well as their chromatographic analysis, *Farmaco*, **1995**, *50*, 245–256.

SAMPLE
Matrix: plants
Sample preparation: 500 mg Plant material + 7 mL MeOH:water 70:30, stir at room temperature for 30 min, centrifuge at 1500 g for 5 min, repeat extraction 3 times. Combine extracts, filter (No. 1 paper), add 2.5 mL 1.172 mg/mL p-tert-octylphenol in MeOH, make up to 25 mL with MeOH:water 70:30, inject a 5 μL aliquot.

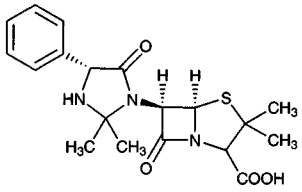
HPLC VARIABLES
Guard column: μBondapak C18
Column: 250 × 4.6 5 μm Cosmosil 5C18 (Nacalai Tesque)
Mobile phase: Gradient. A was MeCN:buffer 10:90. B was MeCN:MeOH:1% acetic acid 45:45:10. A:B from 90:10 to 75:25 over 14 min, maintain at 75:25 for 8 min, to 70:30 over 8 min, to 20:80 over 5 min, to 0:100 over 10 min, maintain at 0:100 over 10 min, return to initial conditions over 5 min. (Buffer contained 20 mM sodium acetate and 419.7 mM acetic acid.)
Flow rate: 0.8
Injection volume: 5
Detector: UV 280

CHROMATOGRAM
Retention time: 28.2
Internal standard: p-tert-octylphenol (54.2)

OTHER SUBSTANCES
Extracted: emodin, gallic acid, honokiol, magnolol, naringin, sennoside A, sennoside B

REFERENCE
Sheu,S.-J.; Lu,C.-F. Determination of eight constituents of Hsiao-cheng-chi-tang by high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *704*, 518–523.

Hetacillin



Molecular formula: $C_{19}H_{23}N_3O_4S$
Molecular weight: 389.48
CAS Registry No.: 3511-16-8, 5321-32-4 (K salt)
Merck Index: 4706
Lednicer No.: 1 414

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 500 μL MeCN:MeOH:water 40:20:40, vortex for 10-15 s, filter (Centricon-10, molecular mass cut-off filter 10000 daltons) with centrifuging at 2677 g for 30 min, inject a 10-100 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 220 × 2.1 5 μm Spheri-5 phenyl microbore (UV detection) or 220 × 4.6 5 μm Spheri-5 phenyl microbore (MS detection)
Mobile phase: MeCN:85% phosphoric acid:triethylamine:water 20:0.4:0.4:79.2 containing 5 mM dodecanesulfonate (UV) or isopropanol:acetic acid in 200 mM ammonium acetate:water 10:2:88 (MS)
Column temperature: 50
Flow rate: 0.2-0.45 (UV) or 0.8-1.2 (MS)

Injection volume: 10-100
Detector: UV 220 or MS, Finnigan MAT 4800 quadrupole, thermospray, source 320°, vaporizer 120°, pulsed positive ion negative ion

CHROMATOGRAM
Retention time: 8.3 (UV), 14.5 (MS) [as ampicillin]
Limit of detection: 200 ng/mL (MS), 75 ng/mL (100)

OTHER SUBSTANCES
Also analyzed: cloxacillin, amoxicillin

KEY WORDS
ultrafiltrate; LC-MS; hetacillin is rapidly converted to ampicillin

REFERENCE
Voyksner,R.D.; Tyczkowska,K.L.; Aronson,A.L. Development of analytical methods for some penicillins in bovine milk by ion-paired chromatography and confirmation by thermospray mass spectrometry, *J.Chromatogr.*, **1991**, *567*, 389–404.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use 18-crown-6 to make the potassium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene: heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

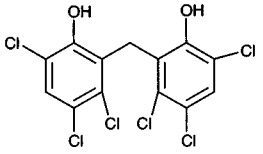
CHROMATOGRAM
Retention time: 7.8

OTHER SUBSTANCES
Simultaneous: carbenicillin, cephapirin, cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin, penicillin G

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

Hexachlorophene



Molecular formula: $C_{13}H_6Cl_6O_2$
Molecular weight: 406.91
CAS Registry No.: 70-30-4
Merck Index: 4716

SAMPLE
Matrix: solutions
Sample preparation: Dissolve 4-20 μg in 1 mL 5% NaOH, add 30 μL p-anisoyl chloride, vortex for 1 min, let stand at room temperature for 20 min, add 9 mL water, vortex for 2 min, extract three times with 10 mL portions of hexane. Combine the extracts and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL butyl chloride, inject a 10 μL aliquot.

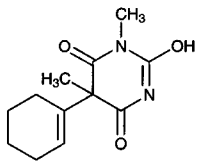
HPLC VARIABLES
Column: 610 × 2.3 36-40 μm Sil-X silica (Nester-Faust)
Mobile phase: Hexane:n-butyl chloride 55:45
Flow rate: 0.7
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 10.5
Limit of detection: 30 ppb

KEY WORDS
derivatization; normal phase

REFERENCE
Porcaro,P.J.; Shubiak,P. Detection of nanogram quantities of hexachlorophene by ultraviolet liquid chromatography, *Anal.Chem.*, **1972**, *44*, 1865–1867.

Hexobarbital



Molecular formula: $C_{12}H_{16}N_2O_3$
Molecular weight: 236.27
CAS Registry No.: 56-29-1, 50-09-9 (sodium salt)
Merck Index: 4742
Lednicer No.: 1 273

SAMPLE
Matrix: solutions

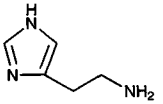
HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC GEL, ODS-AM coated with poly-(R)-1-(α-naphthyl)ethyl methacrylamide (Prepare (R)-1-(α-naphthyl)ethyl methacrylamide by reacting methacryl chloride with (R)-1-(α-naphthyl)ethylamine. Prepare poly-(R)-1-(α-naphthyl)ethyl methacrylamide by polymerizing this compound in anhydrous benzene/THF with 2,2'-azobis(isobutyronitrile) (Caution! Benzene is a carcinogen!). Average molecular weight = 2500. Coat 4 g 5 μm YMC GEL, ODS-AM with 0.8 g of this polymer using dichloromethane as a solvent.)
Mobile phase: MeCN:water 30:70
Flow rate: 0.7

CHROMATOGRAM
Retention time: k' 6.86 (α = 1.08)

KEY WORDS
chiral

REFERENCE
Oi,N.; Hashimoto,S.; Ishizuka,N.; Ohtake,J. Enantiomer separation with poly-(R)-1 (α-naphthyl)-ethyl-methacrylamide coated on ODS silica gel by reversed phase HPLC, *Biomed.Chromatogr.*, **1997**, *11*, 296–297.

Histamine



Molecular formula: $C_5H_9N_3$
Molecular weight: 111.15
CAS Registry No.: 51-45-6, 51-74-1 (phosphate)
Merck Index: 4756

SAMPLE
Matrix: bacterial culture
Sample preparation: Prepare a column by filling a 1 mL pipette tip with 200 mg Extrelut, condition with 250 µL buffer. Add 10 µL bacterial culture and 10 µL IS solution to the column, elute with 1.3 mL ethyl methyl ketone:isopropanol 90:10 (prepared just prior to use) or with 1.3 mL ethyl methyl ketone. Add 200 µL reagent to the eluate, vortex for 15 s, let stand for 30 min, dilute 1 volume of the supernatant with 1 volume of MeOH and 2 volumes of water, inject an aliquot. (Prepare buffer by dissolving 50 mmoles ascorbic acid and 50 mmoles Na_2HPO_4 in 80 mL 1 M NaOH with stirring, adjust pH to 12.5 with 10 mM NaOH, make up to 100 mL with water, store in completely filled vials. Prepare the IS solution by adding 12 µL pentylamine to 100 mL 100 mM HCl. Purify ethyl methyl ketone by passing 20 mL through 4 g acidic aluminum oxide (Merck). Prepare reagent each day by dissolving 10 mg o-phthaldialdehyde and 50 µL ethanethiol in 1 mL MeOH and adding 9 mL 400 mM pH 9.5 sodium borate buffer.)

HPLC VARIABLES
Column: 125 × 2 Superspher 100 RP-18 (Merck)
Mobile phase: MeOH:buffer 85:15 (Buffer was 7% triethylamine adjusted to pH 7.5 with acetic acid.)
Flow rate: 0.2
Injection volume: 1
Detector: F ex 340 em 450

CHROMATOGRAM
Retention time: 2.56
Internal standard: pentylamine (7.45)

OTHER SUBSTANCES
Extracted: cadaverine, isobutylamine, phenethylamine, putrescine, tryptamine, tyramine

KEY WORDS
SPE; derivatization

REFERENCE
Bilic,N. Rapid identification of biogenic amine-producing bacterial cultures using isocratic high-performance liquid chromatography, *J.Chromatogr.A*, **1996**, *719*, 321–326.

SAMPLE
Matrix: beverages

Sample preparation: Condition a 500 mg SAX SPE cartridge (Varian) with two 5 mL portions of MeOH and two 5 mL portions of water. Condition a 1000 mg C18 SPE cartridge with two 5 mL portions of MeOH two 5 mL portions of 200 mM pH 4.5 sodium decanesulfonate. Adjust pH of 15 mL red wine to 8, pass through the SAX SPE cartridge, adjust the pH of the eluate to 4.5, add 100 μL 200 mM sodium decanesulfonate, add this solution to the C18 SPE cartridge, elute with 3 mL MeOH. Mix 10 μL reagent and 2 μL eluate for 1 min, inject the whole amount. (Prepare reagent by dissolving 45 mg o-phthalaldehyde and 200 μL mercaptoethanol in 1 mL MeOH and making up to 10 mL with Buffer. Prepare buffer by dissolving 3.81 g sodium tetraborate in 100 mL water and adjusting pH to 10.5 with 10 M NaOH.)

HPLC VARIABLES

Guard column: ODS Basic (Teknokroma, Barcelona)
Column: 250 × 4.6 5 μm ODS Basic (Teknokroma, Barcelona)
Mobile phase: Gradient. A was THF:50 mM pH 7 (?) sodium acetate 1:99. B was MeOH. A:B from 45:55 to 20:80 over 25 min, maintain at 20:80 for 3 min, return to initial conditions over 2 min.
Column temperature: 60
Flow rate: 1
Injection volume: 12
Detector: F ex 330 em 445

CHROMATOGRAM

Retention time: 4.5
Limit of quantitation: 430 ng/mL

OTHER SUBSTANCES

Extracted: amylamine, butylamine, cadaverine, ethanolamine, ethylamine, hexylamine, isopropylamine, methylamine, 3-methylbutylamine, phenethylamine, propylamine, putrescine, tryptamine, tyramine

KEY WORDS

wine; derivatization; SPE

REFERENCE

Busto,O.; Guasch,J.; Borrull,F. Improvement of a solid-phase extraction method for determining biogenic amines in wines, *J.Chromatogr.A*, **1995**, *718*, 309–317.

SAMPLE

Matrix: blood
Sample preparation: Chill 20 mL whole blood in ice water, add 1 mL reagent, invert several time, centrifuge at 4° at 4000 g for 10 min. Remove the plasma and make it 0.4 M in perchloric acid by adding concentrated perchloric acid, mix, let stand at 4° for 15 min, centrifuge at 4° at 20000 g for 20 min. Remove a 2 mL aliquot and adjust the pH to 7.0 ± 0.2 with 0.5 M KOH, add 400 μL reagent, add 2 g NaCL, add 2 mL ethyl acetate, shake for 1 min, centrifuge at 3400 g, repeat the extraction. Combine the organic layers, add 2 mL 35 mM pH 10.0 ± 0.1 Na_2HPO_4 buffer, shake for 1 min, centrifuge at 3400 g, repeat the wash, evaporate the ethyl acetate layer to 100 μL with a stream of nitrogen, inject a 10-50 μL aliquot. (Prepare reagent as follows. Dissolve 500 mg boric acid in 19 mL water, adjust the pH to 10.40 ± 0.02 with 450 g/L KOH, add 17.5 mg o-phthalaldehyde dissolved in 200 μL MeOH, add 40 μL fresh 2-mercaptoethanol, store under nitrogen at 5°, stable for 7 days (J.Chromatogr. 1979, 162, 293).)

HPLC VARIABLES

Guard column: Co:Pell ODS
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Gradient. MeCN:25 mM pH 5.10 NaH_2PO_4 25:75 for 15 min, then MeOH:25 mM pH 5.10 NaH_2PO_4 45:55 for 35 min (step gradient).
Column temperature: 26
Flow rate: 1.5
Injection volume: 10-50
Detector: F ex 340 em 480

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES
Extracted: dopamine, norepinephrine, octopamine, serotonin, tyramine

KEY WORDS
pig; whole blood; derivatization

REFERENCE
Davis,T.P.; Gehrke,C.W.,Jr.; Williams,C.H.; Gehrke,C.W.; Gerhardt,K.O. Pre-column derivatization and high-performance liquid chromatography of biogenic amines in blood of normal and malignant hyperthermic pigs, *J.Chromatogr.*, **1982**, *228*, 113–122.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with pH 9.7 buffer containing 8.6 mM cyanide and 17.2 mM naphthalene-2,3-dicarboxaldehyde, let stand for 20 min, add 200 mM taurine, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultramex C8
Mobile phase: MeCN:pH 6.8 phosphate buffer 40:60
Detector: F

CHROMATOGRAM
Limit of quantitation: 75 pg

KEY WORDS
derivatization; plasma

REFERENCE
James,J.; Lowe,D.; Karnes,H.T. Determination of histamine from plasma using derivatization with naphthalene-2,3-dicarboxaldehyde and HPLC with fluorescence detection, *Pharm.Res.*, **1992**, *9*, S21.

SAMPLE
Matrix: blood
Sample preparation: Condition a CBA (carboxylic acid) SPE cartridge (Analytichem) with 1 mL MeOH and 2 mL 10 mM pH 7 phosphate buffer. 500 μL Plasma + 100 μL 1 mg/mL betazole + 2 mL ice-cold 10 mM pH 7 phosphate buffer, add to the SPE cartridge at 0.2-0.3 mL/min, dry for 30 s, wash with 1 mL hexane, elute with 1 mL MeOH:100 mM HCl 60:40. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL 200 mM pH 9 sodium borate buffer, add 50 μL 20 μg/mL fluorescamine in MeCN, vortex, store at 4°, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: Pelliguard LC-8 (Supelco)
Column: 250 × 4.6 5 μm Ultramex C8
Mobile phase: MeCN:500 mM pH 7 imidazole buffer 20:80
Flow rate: 1
Injection volume: 50
Detector: F ex 366 em 440

CHROMATOGRAM
Retention time: 15.2
Internal standard: betazole (26.9)
Limit of quantitation: 1 ng/mL

KEY WORDS
plasma; SPE; derivatization

REFERENCE
Lowe,D.R.; March,C.; James,J.E.; Karnes,H.T. A high-performance liquid chromatographic method for histamine in plasma using solid phase extraction and fluorescamine derivatization, *J.Liq.Chromatogr.*, **1994**, *17*, 3563–3570.

SAMPLE
Matrix: blood
Sample preparation: Condition a CBA SPE cartridge with 1 mL MeOH and 2 mL 10 mM pH 7 phosphate buffer. 500 μL Plasma + 2 mL chilled 10 mM pH 7 phosphate buffer, mix, add to the SPE cartridge, dry for 30 min, wash with 1 mL hexane, elute with 1 mL MeOH:100 mM HCl 40:60. Evaporate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL 200 mM pH 9.7 borate buffer. Remove a 10 μL aliquot and add it to 40 μL 20 μg/mL fluorescamine in 200 mM pH 9.7 sodium borate buffer and 50 μL 200 mM pH 9.7 sodium borate buffer, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultramex C-8
Mobile phase: MeCN:500 mM pH 7 imidazole nitrate buffer 20:80
Flow rate: 1
Injection volume: 50
Detector: F ex 366 em 440 (cut-off filter)

CHROMATOGRAM
Limit of detection: 13 pg
Limit of quantitation: 166 pg

KEY WORDS
derivatization; plasma; SPE; details of chemiluminescence detection are also given in paper

REFERENCE
Walters,D.L.; James,J.E.; Vest,F.B.; Karnes,H.T. A comparison of fluorescence versus chemiluminescence detection for analysis of the fluorescamine derivative of histamine by HPLC, *Biomed.Chromatogr.*, **1994**, *8*, 207–211.

SAMPLE
Matrix: blood, food, peptides, plants, tissue
Sample preparation: Hydrolyze peptide with 6 M HCl containing 0.2% 3,3'-thiodipropionic acid at 110° for 24 h, evaporate to dryness, reconstitute with 50-200 μL 0.1% HCl containing 0.2% 3,3'-thiodipropionic acid. Homogenize (Ultra-Turrax) 0.1-1 g food, tissue, plant material, lyophilized plasma, or lyophilized tissue in 10 mL 250 nM IS in 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid at 20000 rpm for 2 min, sonicate for ≤30 min, centrifuge at 5000 g for 20 min, discard fat layer, filter (Millipore ultrafiltration insert (MW cutoff 5000) prewashed with 200 μL 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid) 3 mL supernatant while centrifuging at 3500 g for 1 h. Mix 20 μL deproteinized sample (or 10 μL peptide hydrolysate) with 180 μL buffer, vortex, add 200 μL reagent, mix, heat at 70° for 15 min with mixing at 1 min and 12 min, cool in an ice bath for 5 min, centrifuge at 10000 g for 10 s, add 400 μL diluent, mix thoroughly, centrifuge at 15000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Prepare buffer by dissolving 630 mg sodium bicarbonate in 40 mL water, adjusting pH to 8.6 with NaOH, and making up to 50 mL with water. Prepare reagent by sonicating 40 mg dabsyl chloride in 10 mL acetone for 10 min, then filtering into brown vials and storing at -20°. Prepare diluent by mixing 50 mL MeCN, 25 mL EtOH, and 25 mL mobile phase A.)

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: Gradient. A was DMF:9 mM NaH_2PO_4 containing 0.16% triethylamine, adjusted to pH 6.55 with phosphoric acid. B was MeCN:water 80:20. A:B 92:8 for 2 min, to 80:20 over 5 min (Waters convex curve 5), to 65:35 over 28 min (Waters concave curve 7), to 50:50 over 10 min, to 0:100 over 21 min, maintain at 0:100 for 11 min, return to initial conditions over 0.5 min, re-equilibrate for 12.5 min.
Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 436

CHROMATOGRAM
Retention time: 63.87

Internal standard: norleucine (40.90), norvaline (35.06)

OTHER SUBSTANCES
Extracted: amino acids dopamine, epinephrine, norepinephrine, taurine

KEY WORDS
rinse glass and plasticware with 70% EtOH and water and dry before use; derivatization; cheese; meat; sausage; fish; plasma

REFERENCE
Krause,I.; Bockhardt,A.; Neckermann,H.; Henle,T.; Klostermeyer,H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-performance liquid chromatography of the dabsyl derivatives, *J.Chromatogr.A*, **1995**, *715*, 67–79.

SAMPLE
Matrix: bulk
Sample preparation: Evaporate 100 μL of a solution of histamine in MeOH to dryness under nitrogen, add 100 μL 0.3 mM N-(4-aminobutyl)-N-ethylisoluminol isothiocyanate in MeCN:water:triethylamine 88:10:2 to the residue, vortex for 10 s, heat at 80° for 1 h. Add 600 μL mobile phase to the reaction mixture, mix, inject a 100 μL aliquot. (Synthesis of N-(4-aminobutyl)-N-ethylisoluminol isothiocyanate is as follows. Dissolve 110 mg N-(4-aminobutyl)-N-ethylisoluminol in 500 mL 100 mM sodium carbonate, add 100 μL thiophosgene, stir at room temperature for 2.5 h, adjust pH to 1 with 100 mL 1 M HCl, extract twice with 250 mL portions of ethyl acetate, combine the extracts and evaporate them to dryness under reduced pressure at 30-35°. Dissolve the residue in 4 mL DMF, add 100 mL ice-cold water, store at 4° overnight, collect the precipitate by filtration (0.45 μm), dry under vacuum for 16 h to obtain N-(4-aminobutyl)-N-ethylisoluminol isothiocyanate. Store at 4° in the dark.)

HPLC VARIABLES
Guard column: 10 × 2.0 10 μm PLRP-S (Polymer Labs., UK)
Column: 250 × 4.0 5 μm Asahipak ODP-50 (Hewlett-Packard)
Mobile phase: Gradient. A was MeCN:10 mM pH 10.5 sodium carbonate buffer 30:70 containing 5 mM tetraheptylammonium bromide. B was MeCN:10 mM pH 10.5 sodium carbonate buffer 70:30. A:B 100:0 for 22 min, to 0:100 (step gradient), maintain at 0:100 for 6 min, re-equilibrate at initial conditions for 30 min
Flow rate: 0.8
Injection volume: 100
Detector: E, ESA Coulochem guard cell, Model 5020, porous graphite electrode, Pd/PdO reference electrode

CHROMATOGRAM
Retention time: 20
Limit of detection: 1.5 pmol

KEY WORDS
derivatization

REFERENCE
Steijger,O.M.; Kamminga,D.A.; Brummelhuis,A.; Lingeman,H. Liquid chromatography with luminol-based electrochemiluminescence detection. Determination of histamine, *J.Chromatogr.A*, **1998**, *799*, 57–66.

SAMPLE
Matrix: bulk
Sample preparation: Mix 0.5-5 nmole histamine with 50 nmole luminarin 2 in 500 μL acetone, add 100 μL 100 mM dimethylaminopyridine in acetone, evaporate to dryness, let stand in the dark for 1.5 h,reconstitute with 200 μL acetone, inject an aliquot. (Dry acetone over 0.4 nm molecular sieve. Luminarin 2, N-[6-[(2,5-dioxo-1-pyrrolidinyl)oxy]-6-oxohexyl]-2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-acetamide, may be obtained from Eurobio, Les Ulis, France. Synthesis is as follows. Reflux (with protection from moisture and with stirring) 2.12 g 8-hydroxyjulolidine, 2.22 g diethyl 1,3-acetonedicarboxylate (oxo-3-glutaric acid ethyl ester, Fluka), 1.71 g anhydrous zinc chloride, and 6 mL EtOH for 24 h, cool, add to 200 mL water, extract with 200 mL ethyl acetate, extract with 100 mL ethyl acetate. Combine

the organic layers and wash them with water, dry over magnesium sulfate, evaporate to dryness, recrystallize from 5 parts ethyl acetate to give ethyl 2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-acetate. Heat 2 g of this compound with 42 mL 1.2% NaOH in water and 40 mL MeOH at 45° for 1 h, cool, wash with 50 mL chloroform, wash with 40 mL chloroform. Degas the aqueous phase and acidify it with 16 mL 3 M HCl, stir for 15 min, adjust pH to 6.5 with 13 mL 2.5 M NaOH, filter. Wash the precipitate with water and dry it to obtain 2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-acetic acid. React 30 g potassium carbonate with 30 g methyl 6-aminohexanoate hydrochloride (Fluka) for 30 h, filter. Stir 11.26 g of 2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-acetic acid, 10.62 g disuccinimidyl oxalate (dihydroxysuccinimide carbonate), 3.81 g anhydrous triethylamine, and 560 mL dry MeCN protected from moisture at room temperature for 1 h, stir at 35-40° for 1 h, add the methyl 6-aminohexanoate filtrate, stir for 8 h, add 20 g ethanolamine, stir for 30 min, filter, wash with water, remove the solvent, chromatograph on a silica gel column with dichloromethane, dichloromethane:THF 85:15, dichloromethane:THF 75:25, recrystallize from ethyl acetate to give methyl N-(2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-yl)acetyl-6-aminohexanoate (yield 36%). Heat 1.25 g of this compound with 42 mL 1.2% NaOH in water and 40 mL MeOH at 45° for 1 h, cool, wash with 50 mL chloroform, wash with 40 mL chloroform. Degas the aqueous phase and acidify it with 16 mL 3 M HCl, stir for 15 min, adjust pH to 6.5 with 13 mL 2.5 M NaOH, filter. Wash the precipitate with water and dry it to obtain N-(2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-yl)acetyl-6-aminohexanoic acid. React 750 mg of this acid with 912 mg triethylamine and 512 mg disuccinimidyl oxalate (dihydroxysuccinimide carbonate) in 27 mL MeCN for 6 h, filter, evaporate, chromatograph on a silica gel column with dichloromethane, dichloromethane:THF 85:15, and dichloromethane:THF 75:25 to obtain Luminarin 2 (yield 19%) (World Pat. 89 12,052; Chem. Abstr. 1990, 113, 23889n).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:5 mM ammonium acetate 26:74
Column temperature: 40 (chemiluminescence only)
Flow rate: 2 (F) or 1.5 (chemiluminescence)
Injection volume: 20
Detector: F ex 390 em 490, Chemiluminescence. 1.1 mM Bis(2,4,6-trichlorophenyl) oxalate in methyl acetate pumped at 0.3 mL/min and 400 mM hydrogen peroxide in THF pumped at 0.3 mL/min mixed in a 292 μL capillary tube and this mixture mixed with the column effluent. The resulting mixture flowed through a 60 μL PTFE capillary at 40° to a Kratos FS970 detector fitted with a 470 nm long-pass filter.

CHROMATOGRAM

Retention time: 15.4
Limit of detection: 100 fmole (F), 50 fmole (chemiluminescence)

KEY WORDS

derivatization; comparison with o-phthalaldehyde derivatization

REFERENCE

Tod,M.; Legendre,J.-Y.; Chalom,J.; Kouwatli,H.; Poulou,M.; Farinotti,R.; Mahuzier,G. Primary and secondary amine derivatization with luminarins 1 and 2: separation by liquid chromatography with peroxyoxalate chemiluminescence detection, *J.Chromatogr.*, **1992**, *594*, 386–391.

SAMPLE

Matrix: cell suspensions
Sample preparation: 500 μL Cell suspension in buffer A containing 0.1% bovine serum albumin + 1.5 mL ice cold buffer A, centrifuge at 4° at 250 g for 10 min, remove the supernatant, add 2 mL buffer A to the pellet, boil this mixture for 3 min, cool on ice, centrifuge at 4° at 500 g for 10 min, remove the supernatant. Dry a 50-200 μL aliquot of each supernatant in a vacuum centrifuge and reconstitute the residue with 100 μL buffer B, add 20 μL reagent, inject an aliquot. (Buffer A contained 150 mM NaCl, 4 mM KCl, 1 mM calcium chloride, 1.2 mM magnesium sulfate, 2.46 mM Na_2HPO_4, 0.615 mM KH_2PO_4, 5.6 mM glucose, and 10 mM HEPES, pH 7.4. Buffer B was THF:100 mM pH 9.5 sodium tetraborate buffer 30:70. The reagent was prepared by mixing equal volumes of 3.8 mM o-phthaldialdehyde in MeOH (freshly prepared) and 2.5 mL/L mercaptoethanol in MeOH.)

HPLC VARIABLES
Column: 100 × 3.2 3 μm Phase-II ODS (Bioanalytical Systems)
Mobile phase: MeCN:MeOH:buffer 14:16:70 containing 1 mM disodium EDTA (Buffer was 100 mM sodium phosphate buffer containing 0.4% triethylamine, pH 6.4.)
Flow rate: 0.6
Detector: E, Bioanalytical Systems Model LC4B, LC17A thin-layer electrochemical cell, glassy carbon working electrode +0.5 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 10
Limit of detection: <0.1 pmole

KEY WORDS
rat mast cells; derivatization

REFERENCE
Jensen,T.B.; Marley,P.D. Development of an assay for histamine using automated high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1995**, *670*, 199–207.

SAMPLE
Matrix: cheese, tissue
Sample preparation: Add 1,7-diaminopentane as IS. Homogenize (Polytron) 10 g cheese with two 20 mL portions of 100 mM HCl. Homogenize (Polytron) 10 g tissue with three 15 mL portions of 5% trichloroacetic acid. Saturate the extracts with NaCl, adjust the pH to 11.5. Remove a 5 mL aliquot and add it to 5 mL butanol (cheese) or butanol:chloroform 50:50 (tissue) vortex for 5 min, repeat extraction twice more. Combine the organic extracts and remove a 1 mL aliquot, add 2 drops 1 M HCl, evaporate to dryness under reduced pressure, reconstitute the residue in 1 mL 100 mM HCl, add 0.5 μL saturated sodium bicarbonate, add 1 mL 5 mg/mL dansyl chloride, heat at 40° for 1 h, evaporate to dryness under reduced pressure, reconstitute with MeCN, inject an aliquot.

HPLC VARIABLES
Column: 150 × 1.6 3 μm Spherisorb 3S TG
Mobile phase: Gradient. MeCN:water 65:35 for 1 min, to 80:20 over 4 min, to 90:10 over 1 min.
Flow rate: 0.8
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3.7
Internal standard: 1,7-diaminopentane (4.2)

OTHER SUBSTANCES
Extracted: cadaverine, 2-phenylethylamine, putrescine, spermidine, spermine, tryptamine, tyramine

KEY WORDS
derivatization; fish; salmon; tuna; salami

REFERENCE
Moret,S.; Conte,L.S. High-performance liquid chromatographic evaluation of biogenic amines in foods. An analysis of different methods of sample preparation in relation to food characteristics, *J.Chromatogr.A*, **1996**, *729*, 363–369.

SAMPLE
Matrix: fish, food, wine
Sample preparation: Filter (0.45 μm) wine, fruit juice or vegetable juice, dilute 1:5 or 1:20. 50 μL Diluted wine, diluted juice, fish extract, or cheese extract (neutralize strongly acidic samples if necessary) + 200 μL 200 mM boric acid adjusted to pH 8.5 with 30% KOH + 200 μL 3 mM 9-fluorenylmethyl chloroformate in acetone, mix for 3 min at room temperature, add 50 μL reagent, mix for 3 min. Remove an 80 μL aliquot and add it to 320 μL initial mobile phase,

inject a 20 μL aliquot. (Reagent was 3 mL heptylamine in 15 mL MeCN, adjusted to pH 7-8 with 175 mL 100 mM HCl. Extract fish as follows. Homogenize 20 g fish and 10 mL 100 mM HCl, add 40 mL 100 mM HCl, centrifuge, decant, extract residue with two 40 mL and one 20 mL portions of 100 mM HCl, filter, make up the extracts to 100 (?) mL with 100 mM HCl. Extract cheese as follows. Homogenize 25 g cheese and 18.75 mL 100 mM HCl, suspend with 40 mL 100 mM HCl, centrifuge, extract the residue twice with 20 mL portions of 100 mM HCl. Combine and filter the extracts, make up to 100 mL with 100 mM HCl.)

HPLC VARIABLES

Column: 250-4 Supersphere 60 RP-8 (Merck)
Mobile phase: Gradient. A was MeCN:100 mM pH 4.4 sodium acetate buffer 50:50. B was MeCN. A:B 100:0 for 7 min, to 90:10 over 5 min, to 70:30 over 15 min, maintain at 70:30 for 6 min, to 10:90 over 7 min, to 0:100 over 3 min, maintain at 0:100 for 9 min, return to 100:0 over 1 min, re-equilibrate for 7 min.
Column temperature: 40
Flow rate: 1.2 (0.05 when not in use)
Injection volume: 20
Detector: F ex 265 em 315

CHROMATOGRAM

Retention time: 27.8
Limit of detection: <20 ng/mL

OTHER SUBSTANCES

Extracted: cadaverine, phenylethylamine, putrescine, spermidine, spermine, tyramine

KEY WORDS

wine; fruit juice; vegetable juice; fish; cheese; derivatization

REFERENCE

Kirschbaum,J.; Luckas,B.; Beinert,W.-D. Pre-column derivatization of biogenic amines and amino acids with 9-fluorenylmethyl chloroformate and heptylamine, *J.Chromatogr.A*, **1994**, *661*, 193–199.

SAMPLE

Matrix: food
Sample preparation: Add 10 mL light petroleum to 5 g natural canned tuna or tuna canned in oil, extract, centrifuge at about 13 g for 15 min, discard the organic layer, extract the remaining solid with two or more 10 mL portions of light petroleum. Homogenize the defatted sample with 10 mL 600 mM perchloric acid, filter, add 10 mL 100 mM NaOH, extract with five 25 mL portions of butanol. Combine the organic layers and extract with 20 mL light petroleum and five 10 mL portions of 100 mM HCl, dilute sample to 50 mL with water. Add 1 mL 1 M NaOH to 2 mL aliquot of the diluted sample, let stand for 5 min, add 1 mL derivatizing solution, let stand 5-10 min until the solution reaches a brown color, add 500 mg NaCl, extract with two 3 mL portions of ethyl acetate, centrifuge, evaporate the organic layer under nitrogen to 100 μL, make up to 2 mL with mobile phase, inject a 20 μL aliquot within 30-40 min. (Prepare the derivatizing solution as follows: Mix 1 g sodium tetraborate heptahydrate, 50 mg o-phthalaldehyde, 50 μL β-mercaptoethanol, and 1 mL MeOH, dilute to 50 mL with 1 M NaOH (pH 10).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS2 RP-18
Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH_2PO_4 adjusted to pH 4.0 with 85% H_3PO_4.)
Column temperature: 34-35
Flow rate: 0.8
Injection volume: 20
Detector: F ex 315 em 415

CHROMATOGRAM

Retention time: 7.5
Limit of detection: 5 μg/g

OTHER SUBSTANCES

Extracted: histidine

KEY WORDS
derivatization; tuna; fish

REFERENCE
Frattini,V.; Lionetti,C. Histamine and histidine determination in tuna fish samples using high-performance liquid chromatography. Derivatization with o-phthalaldehyde and fluorescence detection or UV detection of "free" species, *J.Chromatogr.A*, **1998**, *809*, 241–4]245.

SAMPLE
Matrix: food
Sample preparation: Cheese, chocolate. Homogenize (stomacher) 5 g ground cheese or chocolate with 45 mL 70 mM trisodium citrate at 45° for 5 min. Remove a 3 mL aliquot and add it to 3 mL 600 mM trichloroacetic acid, mix, centrifuge at 4° at 10000 g for 10 min, suspend the pellet in 3 mL 300 mM trichloroacetic acid, centrifuge. Combine the supernatants, filter (0.45 μm), make up the filtrate to 10 mL with water, inject an aliquot. Wine. 3 mL Wine + 3 mL 600 mM trichloroacetic acid, centrifuge, filter the supernatant, inject an aliquot of the filtrate. Fish, sauerkraut. Blend 200 g fish or sauerkraut with 200 mL water for 3 min. Remove a 3 mL aliquot and add it to 3 mL 600 mM trichloroacetic acid, centrifuge, filter the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 30 × 3 Corasil C18
Column: 100 × 8 10 μm Nucleosil C18 radial-compression
Mobile phase: DMSO:buffer 47:53 (Prepare mobile phase by dissolving 16 g ninhydrin and 1.2 g hydrindantin in 322 mL DMSO with sonication for 10 min, add 350 mL 1.8 M pH 5.00 sodium acetate buffer, add 2 g sodium dodecyl sulfate dissolved in a mixture of 618 mL DMSO and 710 mL water, flush constantly with nitrogen. Flush column with DMSO:water 50:50 at the end of each day.)
Column temperature: 29
Flow rate: 1
Detector: UV 546 following post-column reaction. The column effluent flowed through a 10 m × 0.25 mm PTFE tube coiled in a figure 8 at 145° to the detector.

CHROMATOGRAM
Retention time: 16
Limit of detection: 800 ng/g (sauerkraut), 300 ng/g (wine)

OTHER SUBSTANCES
Extracted: cadaverine, phenylethylamine, putrescine, tryptamine, tyramine

KEY WORDS
post-column reaction; cheese; chocolate; wine; fish; sauerkraut; tuna

REFERENCE
Joosten,H.M.L.J.; Olieman,C. Determination of biogenic amines in cheese and some other food products by high-performance liquid chromatography in combination with thermo-sensitized reaction detection, *J.Chromatogr.*, **1986**, *356*, 311–319.

SAMPLE
Matrix: food
Sample preparation: Dilute vinegar, wine, or juice 10-fold with water, centrifuge at 2000 g for 15 min. Remove a 20 μL aliquot and mix it with 150 μL buffer and 400 μL 5 mM 2-naphthoxycarbonyl chloride in MeCN, let stand for 3 min, add 50 μL 20 mM glycine in water, let stand for 3 min, add 500 μL MeCN:500 mM pH 4.4 sodium acetate buffer 75:25, mix, inject a 20 μL aliquot. (Prepare buffer by adjusting the pH of 33.43 g boric acid in 950 mL water to 9.0 with 20% KOH, make up to 1 L with water. Synthesis of 2-naphthoxycarbonyl chloride is as follows. Dissolve 30 mmoles 2-naphthol and 30 moles quinoline in 18 g toluene and 5 g dichloromethane, cool to 0°, add 47 mL 1.93 M phosgene in toluene, warm on a steam bath for 10 min, filter, evaporate to remove the solvent, distil the residue (bp 150-152°/9 mm Hg, take up the distillate in ether, crystallize by adding ligroin to obtain 2-naphthoxycarbonyl chloride (mp 66°) (J. Am. Chem. Soc.1951, 73, 2080).)

HPLC VARIABLES
Guard column: present but not specified
Column: 250 × 4.6 4 μm Superspher 60 RP-18e
Mobile phase: Gradient. A was MeCN:100 mM pH 4.4 sodium acetate 40:60. B was MeCN. A:B 77:23 for 7 min, to 65:35 over 31 min, to 30:70 over 3 min, maintain at 30:70 for 5 min, to 0:100 over 1 min, maintain at 0:100 for 6 min, return to initial conditions over 1 min, re-equilibrate for 6 min.
Column temperature: 45
Flow rate: 1.1 for 46 min, to 1.5 over 1 min, maintain at 1.5 for 13 min
Injection volume: 20
Detector: F ex 274 em 335

CHROMATOGRAM
Retention time: 17.15
Limit of detection: 747 ng/g

OTHER SUBSTANCES
Simultaneous: cadaverine, histamine, 2-phenylethylamine, putrescine, spermidine, spermine, tyramine

KEY WORDS
derivatization; vinegar; wine; juice

REFERENCE
Kirschbaum,J.; Busch,I.; Brückner,H. Determination of biogenic amines in food by automated pre-column derivatization with 2-naphthyloxycarbonyl chloride (NOC-Cl), *Chromatographia*, **1997**, *45*, 263–268.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in 10 mM HCl, inject a 1-2 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm 300 Å Nucleosil C8
Mobile phase: MeOH:buffer 15:85 (Buffer was 50 mM pH 3.1 NaH_2PO_4 containing 0.5 mM disodium EDTA and 5 mM sodium 1-pentanesulfonate or sodium 1-octanesulfonate.)
Flow rate: 1
Injection volume: 1-2
Detector: UV 210

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Simultaneous: metabolites, methylhistamine, histidine, methylimidazole acetic acid

REFERENCE
Hermann,K.; Frank,G.; Ring,J. High-performance liquid chromatography for the separation of histamine, its precursor, and metabolites: Application to biological samples, *J.Liq.Chromatogr.*, **1995**, *18*, 189–204.

SAMPLE
Matrix: tears
Sample preparation: 10 μL Tears + 40 μL 200 mM pH 9.1 sodium borate buffer + 50 μL 200 μg/mL fluorescamine in MeCN, mix vigorously, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20-40 μm LiChroprep RP-8
Column: 10 μm Nucleosil C8 or 10 μm RP-8 (Merck)
Mobile phase: MeCN:4 mM pH 3.5 KH_2PO_4 65:35
Flow rate: 0.5
Injection volume: 20
Detector: F ex 390 em 480

CHROMATOGRAM
Retention time: 4
Limit of quantitation: 1 ng/mL

KEY WORDS
derivatization

REFERENCE
Bettero,A.; Angi,M.R.; Moro,F.; Benassi,C.A. Histamine assay in tears by fluorescamine derivatization and high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *310*, 390–395.

SAMPLE
Matrix: tears
Sample preparation: 10 μL Tears + 40 μL 100 mM pH 9.1 borate buffer, mix, add 50 μL 200 μg/mL fluorescamine in MeCN with vigorous stirring, inject a 20 μL aliquot on to column A, divert the fraction containing the derivatized histamine on to column B then remove column A from the circuit (details are sketchy).

HPLC VARIABLES
Column: A 20-40 μm RP-8 (Merck); B 10 μm RP-8 (Merck)
Mobile phase: MeCN:4 mM pH 3.5 phosphate buffer 65:35
Flow rate: 0.5
Injection volume: 20
Detector: F ex 390 em 480

CHROMATOGRAM
Retention time: 4
Limit of detection: 0.1 ng/mL

KEY WORDS
derivatization; column-switching; heart cut

REFERENCE
Bettero,A.; Galiano,F.; Benassi,C.A.; Angi,M.R. A rapid HPLC technique for determining levels of histamine in tears from normal and inflamed human eyes, *Food Chem.Toxicol.*, **1985**, *23*, 303–304.

SAMPLE
Matrix: tissue
Sample preparation: Prepare SPE (A) column by washing 100-200 mesh Dowex 50 W with excess 2 M HCl, with water, with 2 M NaOH, and with water. Equilibrate the resin with 200 mM pH 6.5 sodium phosphate buffer and pack in a 16 × 5 glass column. Prepare SPE (B) column by washing cellulose-phosphate fibrous cation-exchanger (Sigma) in a 13 × 17 glass column with excess 100 mM NaOH, with water, with 100 mM HCl, and with water until the pH reaches 5-6. Homogenize (glass homogenizer) rat brain hypothalamus with 500 μL ice-cold 3% perchloric acid, rinse homogenizer 3 times with 500 μL portions of 3% perchloric acid. Combine the homogenate and rinses, add IS, centrifuge at 4° at 10000 g for 30 min, add the supernatant to SPE column (A), wash with 5 mL water, wash with 4 mL 2 M HCl, elute with 2.5 mL 3.5 M HCl. Evaporate the eluate to dryness, reconstitute the residue in 800 μL water, add 150 μL buffer, add 100 μL 20 mM sulfosuccinimidyl-3-(4-hydroxyphenyl)propionate (sulfo B-H, Pierce) in water, vortex for 30 s, adjust pH to 5.5-6.0 with 100 mM HCl, add to SPE column (B), wash with 5 mL water, wash with 6 mL 1 mM HCl, elute with 2.5 mL 100 mM HCl. Discard the first 500 μL eluate, evaporate the next 200 μL to dryness, reconstitute with 1 volume water, add 9 volumes water to a total volume of 0.11-1 mL, inject a 100 μL aliquot. (Prepare buffer by mixing 100 mM sodium carbonate and 100 mM sodium bicarbonate in a 10:1 ratio.)

HPLC VARIABLES
Guard column: μBondapak C18/Corasil
Column: 250 × 4.6 Ultrasphere ODS
Mobile phase: MeCN:140 mM sodium acetate 73:17 containing 3.89 M (sic) 1-octanesulfonic acid and 56 mg/L (?) EDTA, adjusted to pH 3.48 with glacial acetic acid

Flow rate: 1
Injection volume: 100
Detector: E, ESA Coulochem 5100A, Model 5011 analytical cell, cell 1 0.47 V, cell 2 (monitored) 0.56 V, oxidative screen mode

CHROMATOGRAM
Retention time: 8.4
Internal standard: N^{α}-methylhistamine (11.6)
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Extracted: N^{tau}-methylhistamine
Simultaneous: histidine, spermidine

KEY WORDS
derivatization; rat; brain; SPE

REFERENCE
Mine,K.; Jacobson,K.A.; Kirk,K.L.; Kitajima,Y.; Linnoila,M. Simultaneous determination of histamine and N tau-methylhistamine with high-performance liquid chromatography using electrochemical detection, *Anal.Biochem.*, **1986**, *152*, 127–135.

SAMPLE
Matrix: tissue
Sample preparation: Sonicate 10 g tuna with 50 mL 1200 ppm triethylamine in MeOH for 10 min, filter, filter (0.45 μm) again, add 25 μL filtrate to 10 mg 3,5-dinitrobenzoyl derivatized silica, heat at 60° for 10 min, elute with 1 mL MeCN, inject a 20 μL aliquot of the eluate. (Prepare 3,5-dinitrobenzoyl derivatized silica as follows. Heat 4.7 g 4-hydroxy-3-nitrobenzoic acid, 7.5 mL thionyl chloride, 750 μL pyridine, and 60 mL benzene (Caution! Benzene is a carcinogen!) at 55° for 4 h, cool to room temperature, filter, evaporate under reduced pressure to obtain 4-hydroxy-3-nitrobenzoyl chloride. Store 10 μm LiChrosorb Si-100 silica in a desiccator over saturated aqueous LiCl solution for 2 weeks. 5 g Silica + 8.4 g 62% bis(2-hydroxyethyl)-3-aminopropyltriethoxysilane (Petrarch Systems, Bristol PA) in EtOH, add 40 mL EtOH:pyridine 99.5:0.5 (?), reflux with stirring under nitrogen for 6 h, cool, filter, wash silica with three 25 mL portions of MeOH and four 25 mL portions of dichloromethane, dry under nitrogen at 40° overnight. Heat 5 g 4-hydroxy-3-nitrobenzoyl chloride, 300 μL pyridine, 5 g silica, and 60 mL benzene (previously dried over anhydrous sodium sulfate) at 70-75° for 2 h, filter, wash silica with three 75 mL portions of DMF and three 75 mL portions of dichloromethane, dry under vacuum at 40° for 12 h. Stir 400 mg silica, 600 mg 3,5-dinitrobenzoyl chloride, 25 mL benzene, and 300 μL pyridine at room temperature for 24 h, filter, wash with three 25 mL portions of dichloromethane to give 3,5-dinitrobenzoyl derivatized silica.)

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosphere C18
Mobile phase: MeCN:water 50:50 containing 0.05% ammonium hydroxide
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2
Limit of detection: 152 ppb

KEY WORDS
derivatization; tuna; fish

REFERENCE
Zhou.F.-X.,; Wahlberg,J.; Krull,I.S. Silica based 3,5-dinitrobenzoyl (DNB) reagent for off-line derivatization of amine nucleophiles in HPLC, *J.Liq.Chromatogr.*, **1991**, *14*, 1325–1350.

SAMPLE
Matrix: tissue

Sample preparation: Incubate chick embryo retinas in 4 mL medium at 37° for 4 h, centrifuge at 500 g for 1 min. For each 20 mg solid add 1 mL 20 μM 1,7-diaminoheptane in 200 mM perchloric acid, suspend, sonicate (Soniprep 150) with 10 μm amplitude in 10 s bursts, centrifuge at 20000 g for 15 min, neutralize with KOH, centrifuge. Remove a 1 mL aliquot and add it to 1 mL 2 M NaOH, add 5 μL benzoyl chloride, vortex briefly, let stand for 20 min, add 2 mL saturated NaCl, extract with 2 mL diethyl ether, centrifuge. Remove the upper organic phase and wash it with 2 mL 100 μM NaOH, dry the organic layer over a few mg anhydrous sodium sulfate, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 2 mL mobile phase, inject a 20 μL aliquot. (Medium was pH 7.4 serum- and glutamine-free Eagle's minimum essential medium containing 25 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES), 100 U/mL penicillin, and 100 μg/mL streptomycin.)

HPLC VARIABLES

Column: 150 × 4.6 3 μm Spherisorb ODS2
Mobile phase: MeOH:water 62:38
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 10.35
Internal standard: 1,7-diaminoheptane (5.4)
Limit of quantitation: 1.25 μM

OTHER SUBSTANCES

Extracted: N-acetylcadaverine, N-acetylputrescine, N^1-acetylspermidine, N^1-acetylspermine, cadaverine, putrescine, spermidine, spermine

KEY WORDS

derivatization; retina; chicken

REFERENCE

Taibi,G.; Schiavo,M.R. Simple high-performance liquid chromatographic assay for polyamines and their monoacetyl derivatives, *J.Chromatogr.*, **1993**, *614*, 153–158.

SAMPLE

Matrix: tissue
Sample preparation: Make a slurry of 40 μm Bakerbond carboxylic acid material in MeOH and prepare SPE columns by adding an aliquot containing 150 mg material to a Pasteur pipette plugged with glass fiber prefilter material (Sartorius). Wash column with 2 mL water, 2 mL 1 M HCl, 2 mL water, and 4 mL 200 mM pH 6.4 sodium phosphate buffer. Homogenize (Ultra-Turrax) rat heart and 500 ng 1-methylhistamine in ice-cold 50 mM pH 8.5 Tris-HCl buffer, sonicate (MSE sonifier, 12 W, 12 μm peak-peak) for 2 min with repeated intervals of 5 s, add perchloric acid to a final concentration of 300 mM, heat at 100° for 5 min, neutralize with KOH, cool to 0°, centrifuge at 4° at 2000 g for 5 min, adjust pH of supernatant to 6.4 with phosphate buffer, add 1-methylhistamine, add to the SPE column, wash with 4 mL 50 mM pH 6.4 disodium EDTA, wash with 4 mL water, elute with 1 mL 1 M HCl. Evaporate the eluate to dryness under a stream of nitrogen at 40° over about 15 min, reconstitute with 100 μL water, add 400 μL 50 mM pH 9.1 sodium borate, with continuous vigorous stirring add 500 μL 200 μg/mL fluorescamine in MeCN (freshly prepared), stir for 1 min, evaporate to dryness under a stream of nitrogen at 40°, reconstitute with 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 200 × 3 5 μm Inertsil ODS-2 (see J. Chromatogr. B 1994, 657, 261)
Mobile phase: MeCN:MeOH:water:phosphoric acid 15:10:75:0.2 adjusted to pH 6.87 with ammonium hydroxide (see J. Chromatogr. B 1994, 657, 261)
Column temperature: 20
Flow rate: 0.4
Injection volume: 20
Detector: F ex 360 em 440

CHROMATOGRAM
Retention time: 15
Internal standard: 1-methylhistamine (25)
Limit of detection: 20 pg

OTHER SUBSTANCES
Extracted: 3-methylhistamine

KEY WORDS
SPE; derivatization; rat; heart

REFERENCE
van Haaster,C.M.C.J.; Engels,W.; Lemmens,P.J.M.R.; Hornstra,G.; van der Vusse,G.J. Rapid and highly sensitive high-performance liquid chromatographic method for the determination of histamine and 3-methylhistamine in biological samples using fluorescamine as the derivatizing agent, *J.Chromatogr.*, **1993**, *617*, 233–240.

SAMPLE
Matrix: tissue
Sample preparation: Blend 50 g tissue and 75 mL 5% trichloroacetic acid in a Waring Blendor at high speed for 2 min, centrifuge for 2 min, extract the solid twice more with 75 mL portions of 5% trichloroacetic acid, filter the supernatants through a glass wool plug, wash the funnel with 5% trichloroacetic acid, make up the filtrate to 250 mL with 5% trichloroacetic acid. Remove a 10 mL aliquot and add it to 4 g NaCl, 1 mL 50% NaOH, and 5 mL chloroform:n-butanol 50:50, shake vigorously for 2 min, centrifuge for 5 min, remove the upper organic layer, repeat the extraction twice more with 5 mL portions of chloroform:n-butanol 50:50. Combine the organic layers and add them to 15 mL n-heptane, extract three times with 1 mL portions of 200 mM HCl, inject a 20 μL aliquot (J.Assoc.Off.Anal.Chem. 1978, 61, 139).

HPLC VARIABLES
Column: PRP-X200 Cationic (Hamilton)
Mobile phase: 400 mM pH 4.5 KH_2PO_4
Flow rate: 0.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 2
Limit of detection: 10 ng

KEY WORDS
fish

REFERENCE
Kalligas,G.; Kaniou,I.; Zachariadis,G.; Tsoukali,H.; Epivatianos,P. Thin layer and high pressure liquid chromatographic determination of histamine in fish tissues, *J.Liq.Chromatogr.*, **1994**, *17*, 2457–2468.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 200 mg tissue with 5 volumes of 1 M perchloric acid, centrifuge at 10000 g for 20 min. Neutralize the supernatant with 10 M KOH, centrifuge at 10000 g for 20 min, remove a 20 μL aliquot of the supernatant and add it to 10 μL reagent, mix well, add 30 μL 10% sodium carbonate in EtOH:water 5:95, mix well, inject a 10 μL aliquot. (Prepare reagent prior to use by mixing equal volumes of 20 mM sulfanilic acid in 1 M HCl and 200 mM sodium nitrite solution.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-ODS (Toso)
Mobile phase: Gradient. A was EtOH:150 mM pH 6.0 sodium acetate buffer 5:95. B was MeCN:water 60:40. A:B from 100:0 to 55:45 over 30 min.
Column temperature: 40

Flow rate: 1
Injection volume: 10
Detector: UV 420

CHROMATOGRAM
Retention time: 14.58
Limit of detection: <7 nmole/g

OTHER SUBSTANCES
Extracted: carnosine, histidine, tyrosine
Noninterfering: anserine, 1-methylhistidine

KEY WORDS
derivatization; fish; muscle; mackerel

REFERENCE
Sato,M.; Nakano,T.; Takeuchi,M.; Kumagai,T.; Kanno,N.; Nagahisa,E.; Sato,Y. Specific determination of histamine in fish by high-performance liquid chromatography after diazo coupling, *Biosci.Biotechnol.Biochem.*, **1995**, *59*, 1208–1210.

SAMPLE
Matrix: tissue
Sample preparation: 10 g Homogenized fish + 15 mL 600 mM perchloric acid, stir magnetically for 10 min, centrifuge at 3000 rpm for 10 min, remove the supernatant, add 10 mL 600 mM perchloric acid to the residue, stir magnetically for 10 min, centrifuge at 3000 rpm for 10 min. Combine the supernatants, make up to 25 mL with 600 mM perchloric acid, filter (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: Gradient. A was 100 mM sodium acetate containing 10 mM sodium octanesulfonate, adjusted to pH 5.20 with acetic acid. B was MeCN:buffer 66:34 (Buffer was 200 mM sodium acetate containing 10 mM sodium octanesulfonate, adjusted to pH 4.50 with acetic acid.) A:B from 80:20 to 20:80 over 50 min, maintain at 20:80 for 2 min, return to initial conditions over 2 min, re-equilibrate for 10 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 445 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min, the mixture flowed through a 200 cm × 0.25 mm i.d. coil of stainless steel tubing to the detector. (Prepare reagent by dissolving 15.5 g boric acid and 13.1 g KOH in 500 mL water, adjust pH to 10.5-11 with 30% KOH (if necessary), add 1.5 mL 30% Brij-35, add 1.5 mL mercaptoethanol, add 2.5 mL 40 μg/mL o-phthalaldehyde in MeOH, mix. Protect from light, prepare fresh daily.)

CHROMATOGRAM
Retention time: 35
Limit of detection: 250 ng/g

OTHER SUBSTANCES
Extracted: agmatine, cadaverine, creatinine, β-phenylethylamine, putrescine, serotonin, spermidine, spermine, tryptamine, tyramine

KEY WORDS
post-column reaction; fish

REFERENCE
Veciana-Nogues,M.T.; Hernandez-Jover,T.; Marine-Font,A.; Vidal-Carou,M.D.C. Liquid chromatographic method for determination of biogenic amines in fish and fish products, *J.AOAC Int.*, **1995**, *78*, 1045–1050.

SAMPLE
Matrix: tissue

Sample preparation: Homogenize (Ultraturrax) 5 g fish, 10 mL 200 mM perchloric acid, and 100 μL 800 μg/mL 1,3-diaminopropane dihydrochloride in water at -20° and 20000 rpm and centrifuge at 2° at 2500 g for 20 min. Remove a 100 μL aliquot of the supernatant and add it to 200 μL saturated sodium bicarbonate solution, add 400 μL 7.5 mg/mL dansyl chloride in acetone, agitate, heat at 60° in the dark for ?, add 100 μL 100 mg/mL L-proline in water, agitate, let stand in the dark at room temperature for 30 min, add 500 μL toluene, agitate. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL MeCN, filter, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Brownlee C18
Column: 250 × 4.6 5 μm Kromasil C18
Mobile phase: Gradient. MeCN:water from 60:40 to 75:25 over 6 min, maintain at 75:25 for 2 min, to 95:5 over 5 min, maintain at 95:5 for 7 min, re-equilibrate at initial conditions for 10 min.
Column temperature: 25
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 14.7
Internal standard: 1,3-diaminopropane (12.5)

OTHER SUBSTANCES

Extracted: cadaverine, putrescine, spermidine, spermine

KEY WORDS

derivatization; fish

REFERENCE

Malle,P.; Vallé,M.; Bouquelet,S. Assay of biogenic amines involved in fish decomposition, *J.AOAC Int.*, **1996**, *79*, 43–49.

SAMPLE

Matrix: urine, blood
Sample preparation: Prepare a 500 μL SPE cartridge of Amberlite CG-50 resin and wash it with 2 mL water. Add urine or blood to the SPE cartridge, wash with 2 mL water, wash with two 2 mL portions of 500 mM pH 6.5 sodium acetate, wash with two 2 mL portions of water, elute with 400 μL 2 M perchloric acid, elute with two 1 (urine) or 0.2 (blood) mL portions of mobile phase, inject an aliquot of the eluate.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:100 mM KH_2PO_4 25:75 containing 56 μg/mL sodium dodecyl sulfate and 3.2 mL/L 10 M NaOH
Flow rate: 1.2
Detector: E, ESA model 5100A, detector 1 +0.85 V, detector 2 +1.12 V, guard cell +1.15 V

CHROMATOGRAM

Retention time: 11
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: N-methylhistamine

KEY WORDS

guinea pig; whole blood; SPE

REFERENCE

Houdi,A.A.; Crooks,P.A.; Van Loon,G.R.; Schubert,C.A. A simple and sensitive determination of histamine and N^{tau}-methylhistamine in biological fluids by high-performance liquid chromatography with electrochemical detection, *J.Pharm.Sci.*, **1987**, *76*, 398–401.

SAMPLE
Matrix: wine
Sample preparation: 20 μL Wine + 50 μL buffer, mix, add 100 μL 8 mg/mL 9-fluorenylmethyl chloroformate in MeCN, mix, let stand for 3 min, add 50 μL 500 mM ammonia, mix, let stand for 3 min, add 300 μL MeCN:water:acetic acid 80:12:8, inject a 20 μL aliquot. (Prepare buffer by adjusting the pH of 200 mM boric acid to 8.5 with 5 M NaOH.)

HPLC VARIABLES
Column: 200 × 2.1 5 μm ODS Hypersil
Mobile phase: Gradient. A was MeCN:2-octanol 99:1. B was MeCN:water:phosphoric acid:dimethylcyclohexylamine 15:83.12:0.88:1, pH 2.7. A:B 15:85 for 18 min, 38:62 over 0.1 min, to 40:60 over 6.9 min, maintain at 40:60 for 5 min, to 42:58 over 37 min, to 85:15 over 36 min, maintain at 85:15 for 7 min.
Flow rate: 0.3
Injection volume: 2
Detector: F ex 263 em 313

CHROMATOGRAM
Retention time: 100

OTHER SUBSTANCES
Extracted: agmatine, arginine, cadaverine, histidine, ornithine, phenylalanine, phenylethylamine, putrescine, spermidine, spermine, tyramine, tyrosine

KEY WORDS
derivatization

REFERENCE
Bauza,T.; Blaise,A.; Daumas,F.; Cabanis,J.C. Determination of biogenic amines and their precursor amino acids in wines of the Vallée du Rhône by high-performance liquid chromatography with precolumn derivatization and fluorimetric detection, *J.Chromatogr.A*, **1995**, *707*, 373–379.

SAMPLE
Matrix: wine
Sample preparation: Wash 5 g Amberlite CG-50 with water, add 5 mL 10 M NaOH, let stand for 30 min, rinse with water 3 times, add 25 mL 5 M HCl to a pH of 2, wash, mix with 5 mL 10 M NaOH, wash with 1 volume pH 7 buffer. Filter (0.45 μm) wine, add 10 mL to the resin in a 40 × 10 column, wash with water, elute with 10 mL 1 M HCl. Evaporate the eluate to 1 mL under reduced pressure, add heptylamine, derivatize with reagent, inject an aliquot. (Reagent was 45 mg phthalaldehyde in 1 mL MeOH, add 200 μL 2-mercaptoethanol, make up to 10 mL with buffer, prepare daily. Buffer was 3.81 g sodium tetraborate in 100 mL water, adjust to pH 10.5 with 10 M NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: Gradient. A was THF:water 1:99 containing 0.03% triethanolamine. B was MeOH. A:B from 40:60 to 20:80 over 25 min, re-equilibrate for 3 min. (Every 10 analyses flush with MeCN:80.8 mM acetic acid 30:70 for 1 h.)
Column temperature: 60
Flow rate: 1
Detector: F ex 330 em 445

CHROMATOGRAM
Retention time: 4.5
Internal standard: heptylamine (21.5)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: biogenic amines

KEY WORDS
derivatization; SPE

REFERENCE
Busto,O.; Mestres,M.; Guasch,J.; Borrull,F. Determination of biogenic amines in wine after clean-up by solid-phase extraction, *Chromatographia*, **1995**, *40*, 404–410.

SAMPLE
Matrix: wine
Sample preparation: Mix 10 µL wine with 6 µL pH 8.8 borate buffer (Waters), add 0.5 µL 10 mM 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate in MeCN (Waters), mix, let stand for 5 min, inject a 10 µL aliquot.

HPLC VARIABLES
Guard column: Spherisorb ODS-2
Column: 250 × 4.6 5 µm Spherisorb ODS-2
Mobile phase: Gradient. A was THF:50 mM sodium acetate. B was MeOH. A:B 75:25 for 5 min, to 20:80 over 20 min, to 0:100 (step gradient), maintain at 0:100 for 3 min, return to initial conditions, re-equilibrate for 2 min.
Column temperature: 65
Flow rate: 1
Injection volume: 10
Detector: F ex 250 em 395

CHROMATOGRAM
Retention time: 9
Internal standard: heptylamine
Limit of detection: 100-500 ng/mL

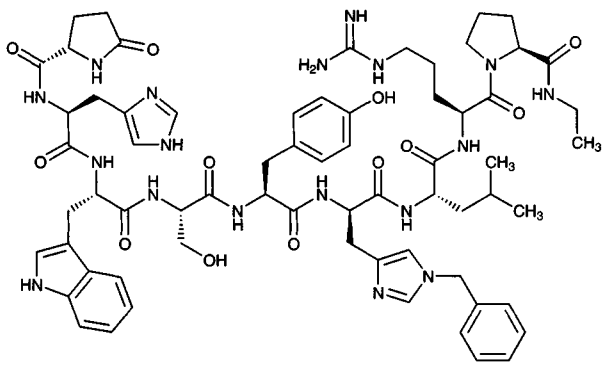
OTHER SUBSTANCES
Extracted: ammonia, amylamine, butylamine, cadaverine, ethanolamine, ethylamine, hexylamine, isopropylamine, methylamine, 3-methylbutylamine, phenethylamine, propylamine, putrescine, pyrrolidine, tyramine

KEY WORDS
derivatization

REFERENCE
Busto,O.; Guasch,J.; Borrull,F. Determination of biogenic amines in wine after precolumn derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, *J.Chromatogr.A*, **1996**, *737*, 205–213.

Histrelin

Molecular formula: $C_{66}H_{86}N_{18}O_{12}$
Molecular weight: 1323.52
CAS Registry No.: 76712-82-8
Merck Index: 4760



SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 µm SP-150 C8 (DuPont)

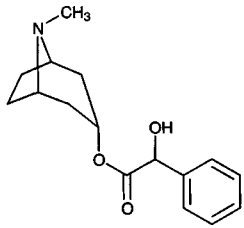
Mobile phase: Gradient. A was 654 mL triethylamine, 444 mL 85% phosphoric acid, and 29.1 g sodium butanesulfonate in 8 L water, pH 2.4. B was MeCN containing enough ethyl acetate to make the absorbance at 210 nm the same as that of mobile phase A. A:B from 85:15 to 78:22 over 25 min (Perkin-Elmer concave curve 4), maintain at 78:22 for 10 min
Flow rate: 1.6
Injection volume: 50
Detector: UV 210, UV 280

CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Oyler,A.R.; Naldi,R.E.; Lloyd,J.R.; Graden,D.A.; Shaw,C.J.; Cotter,M.L. Characterization of the solution degradation products of histrelin, a gonadotropin releasing hormone (LH/RH) agonist, *J.Pharm.Sci.*, **1991**, *80*, 271–275.

Homatropine



Molecular formula: $C_{16}H_{21}NO_3$
Molecular weight: 275.35
CAS Registry No.: 87-00-3, 51-56-9 (HBr)
Merck Index: 4766

SAMPLE
Matrix: plants
Sample preparation: Dissolve alkaloids in 1 mL MeOH, inject aliquot.

HPLC VARIABLES
Column: 150 × 4.1 5 μm Hamilton PRP-1
Mobile phase: MeCN:100 mM pH 10.4 ammonium acetate
Flow rate: 1
Injection volume: 20
Detector: MS thermospray, VG Trio-2, ion source 150°, vaporizer tip 170°, repeller electrode 150 V, m/z 276

CHROMATOGRAM
Internal standard: homatropine
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: hyoscyamine, scopolamine

KEY WORDS
total run time 6 min; homatropine is IS

REFERENCE
Auriola,S.; Martinsen,A.; Oksman-Caldentey,K.M.; Naaranlahti,T. Analysis of tropane alkaloids with thermospray high-performance liquid chromatography-mass spectrometry, *J.Chromatogr.*, **1991**, *562*, 737–744.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 15:1.5:0.5:83
Flow rate: 1.5
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: scopolamine, methscopolamine, tropic acid, atropine methyl, atropine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH or water to 0.1%.

HPLC VARIABLES
Column: two 250 mm β-cyclodextrin bonded phase columns in series (Advanced Separation Technologies)
Mobile phase: MeOH:1% pH 4.1 aqueous triethylammonium acetate 4:96
Flow rate: 0.5
Injection volume: 1
Detector: UV

CHROMATOGRAM
Retention time: k' 1.98 (d-isomer)

KEY WORDS
chiral; optical isomers are separated

REFERENCE
Armstrong,D.W.; Han,S.M.; Han,Y.I. Separation of optical isomers of scopolamine, cocaine, homatropine, and atropine, *Anal.Biochem.*, **1987**, *167*, 261–264.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: k' 0.84 (of first (+) enantiomer)

KEY WORDS
chiral; α 3.13

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.77 (A), 3.63 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

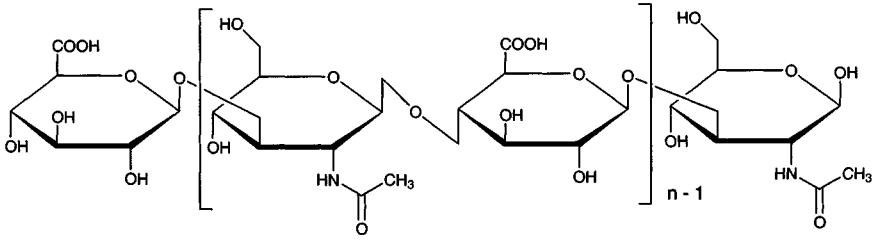
details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Hyaluronic acid

CAS Registry No.: 9004-61-9
Merck Index: 4793



SAMPLE

Matrix: bulk

Sample preparation: Mix 100 μL of a 1-500 μg/mL solution of hyaluronic acid in water with 40 μL 100 mM pH 5.2 citrate/phosphate buffer, add 10 500 U/mL hyaluronate 4-glycanohydrolase (sheep testis, Type V, Sigma) in water, heat at 37° for 5 h, heat in a boiling water bath for 3 min, evaporate to dryness. Reconstitute with 20 μL 300 mM NaOH, add 20 μL 500 mM 1-(4-methoxy)phenyl-3-methyl-5-pyrazolone in MeOH, heat at 70° for 20 min, add 20 μL 300 mM HCl, add 200 μL water, add 200 μL ethyl acetate saturated with water, shake vigorously, discard the organic phase, repeat the ethyl acetate wash twice more. Evaporate the aqueous phase to dryness and reconstitute the residue in 200 μL MeCN:water 15:85, inject a 20 μL aliquot. (Synthesis of 1-(4-methoxy)phenyl-3-methyl-5-pyrazolone is as follows. Reflux 5.6 g 4-methoxyphenylhydrazine hydrochloride, 5.45 g sodium acetate trihydrate, and 4.16 g ethyl acetoacetate in 40 mL EtOH for 2 h, cool, evaporate to dryness, dissolve the residue in 10 mL EtOH, filter, evaporate the filtrate to dryness, dissolve the residue in a small volume of benzene:ethyl acetate 80:20 (Caution! Benzene is a carcinogen!), chromatograph on a column of 150 g silica gel 60 (Merck) equilibrated with benzene:ethyl acetate 80:20, collect 5 mL fractions (monitor by TLC using Merck silica gel 60 F_{254} eluted with benzene:ethyl acetate 80:20, UV detection, R_f 0.41). Combine the appropriate fractions and evaporate them to dryness, recrystallize the residue from MeOH to give 1-(4-methoxy)phenyl-3-methyl-5-pyrazolone (Anal. Biochem. 1991, 199, 256).)

HPLC VARIABLES

Column: 150 × 6 Cosmosil 5C18-AR
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 15:85
Flow rate: 0.8
Injection volume: 20
Detector: UV 249

CHROMATOGRAM

Retention time: 9 (hexasaccharide), 10 (tetrasaccharide), 16 (disaccharide)

KEY WORDS

derivatization

REFERENCE

Kakehi,K.; Ueda,M.; Suzuki,S.; Honda,S. Determination of hyaluronic acid by high-performance liquid chromatography of the oligosaccharides derived therefrom as 1-(4-methoxy)phenyl-3-methyl-5-pyrazolone derivatives, *J.Chromatogr.*, **1993**, *630*, 141–146.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1.5 mg/mL solution of sodium hyaluronate in 0.9% saline, filter 0.22 μm, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: TSK G6000 PW (Toyo Soda)

Column: 300 × 7.5 TSK G6000 PW (Toyo Soda)
Mobile phase: 3 mM pH 7.0 NaH_2PO_4 containing 150 mM NaCl and 0.02% sodium azide
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM
Retention time: 8.22

REFERENCE
Beaty,N.B.; Tew,W.P.; Mello,R.J. Relative molecular weight and concentration determination of sodium hyaluronate solutions by gel-exclusion high-performance liquid chromatography, *Anal.Biochem.*, **1985**, *147*, 387–395.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 8 Shodex OHpak KB-803 (Showa Denko)
Mobile phase: 200 mM NaCl
Flow rate: 0.5
Detector: UV 210

CHROMATOGRAM
Retention time: 12

KEY WORDS
GPC

REFERENCE
Nakamura,T.; Majima,M.; Kubo,K.; Takagaki,K.; Tamura,S.; Endo,M. Hyaluronidase assay using fluorogenic hyaluronate as a substrate, *Anal.Biochem.*, **1990**, *191*, 21–24.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 TSKgel NH2-60
Mobile phase: MeCN:buffer 54:46 (Buffer was 40 mM Tris-HCl borate buffer adjusted to pH 7.5 with HCl containing 5 mM sodium sulfate.)
Flow rate: 0.5
Injection volume: 10-20
Detector: F ex 346 em 410 following post-column reaction. The effluent from the column was mixed with 300 mM NaOH (pumped at 0.25 mL/min) and 1% 2-cyanoacetamide (pumped at 0.25 mL/min). The mixture passed through a 10 m × 0.5 mm i.d. PTFE coil at 105° and a 2 m × 0.25 mm i.d. PTFE coil at 25° to the detector.

CHROMATOGRAM
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: chondroitin sulfate, dermatan sulfate

KEY WORDS
post-column reaction

REFERENCE
Akiyama,H.; Saito,M.; Qiu,G.; Toida,T.; Imanari,T. Analytical studies on hyaluronic acid synthesis by normal human epidermal keratinocytes cultured in a serum-free medium, *Biol.Pharm.Bull.*, **1994**, *17*, 361–364.

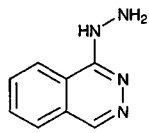
SAMPLE
Matrix: synovial fluid
Sample preparation: Centrifuge synovial fluid, dilute 40 μL to 500 μL with initial mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: LC 18 (Supelco)
Column: 50 × 4.6 Supelcosil LC 318
Mobile phase: Gradient. A was 20 mM NaH_2PO_4 containing 150 mM NaCl, pH 6.5. B was MeCN. A:B from 100:0 to 40:60 over 50 min.
Flow rate: 1
Detector: UV 229

CHROMATOGRAM
Retention time: 0.6

REFERENCE
Brun,P.; De Galateo,A.; Camporese,A.; Cortivo,R.; Abatangelo,G. Analysis of hyaluronic acid in synovial fluid by reversed-phase liquid chromatography, *J.Chromatogr.*, **1990**, *526*, 530–534.

Hydralazine



Molecular formula: $C_8H_8N_4$
Molecular weight: 160.18
CAS Registry No.: 86-54-4, 304-20-1 (HCl)
Merck Index: 4800

SAMPLE
Matrix: blood
Sample preparation: Add 8-12 mL blood to 125 IU lithium heparin in ice-cold tubes, centrifuge at 8000 g for 30 s, add 1 mL plasma to 75 μL 50% sodium nitrite in a tube kept on ice, add 150 μL 16.7 μM 4-methylhydralazine in 10 mM HCl, add 2 mL 20 mM HCl (perform the preceding procedure as rapidly as possible), vortex briefly, allow to stand at 20 ± 1° for 10.0 min, add 1 mL 1 M NaOH/0.6 M sodium tetraborate buffer (pH 10), add chloroform, shake at 110 rpm for 5 min, centrifuge at 1100 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 10 μm μBondapak phenyl
Mobile phase: MeCN:1.5 mM aqueous phosphoric acid 15:85
Column temperature: 50
Flow rate: 2
Injection volume: 50
Detector: F ex 250 em 360 (cut-off filter)

CHROMATOGRAM
Retention time: 6.7
Internal standard: 4-methylhydralazine (10)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, propranolol, quinidine

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE

Reece,P.A.; Cozamanis,I.; Zacest,R. Selective high-performance liquid chromatographic assays for hydralazine and its metabolites in plasma of man, *J.Chromatogr.*, **1980**, *181*, 427–440.

SAMPLE

Matrix: blood

Sample preparation: 3 mL Whole blood + 20 μL p-anisaldehyde + 8 μL 5 μg/mL 4-methylhydralazine in 10 mM HCl, vortex for 15 s, let stand at room temperature for 10 min, add 10 mL hexane, shake horizontally at 180 strokes/min for 10 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject the whole amount.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak CN
Mobile phase: MeCN:150 mM pH 3.0 sodium acetate buffer 70:30
Flow rate: 2
Injection volume: 100
Detector: UV 365

CHROMATOGRAM

Retention time: 3.5
Internal standard: 4-methylhydralazine (6)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Noninterfering: propranolol, furosemide, hydrochlorothiazide, digoxin, nitroglycerin

KEY WORDS

whole blood; derivatization

REFERENCE

Ludden,T.M.; Ludden,L.K.; Wade,K.E.; Allerheiligen,S.R. Determination of hydralazine in human whole blood, *J.Pharm.Sci.*, **1983**, *72*, 693–695.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL 20 mM disodium EDTA + 1 mL 500 mM HCl + 1 mL 10 mM 2-hydroxy-1-naphthaldehyde, vortex for 15 s, keep at 25° for 90 min, add 50 μL 1 μg/mL methyl red, add 7 mL dichloromethane, vortex for 5 min, centrifuge at 4500 rpm for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL MeCN, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 3 μm Spherisorb ODS-2
Mobile phase: MeCN:buffer 80:20, pH 3 (Buffer was 0.75% phosphoric acid and 0.5% triethylamine in water.)
Flow rate: 0.7
Injection volume: 20
Detector: UV 406

CHROMATOGRAM

Retention time: 6.4
Internal standard: methyl red (6)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: dihydralazine

KEY WORDS

plasma; derivatization; pharmacokinetics

REFERENCE
Mañes,J.; Mari,J.; Garcia,R.; Font,G. Liquid chromatographic determination of hydralazine in human plasma with 2-hydroxy-1-naphthaldehyde pre-column derivatization, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 795–798.

SAMPLE
Matrix: formulations
Sample preparation: Injections. Dilute 1.5 mL of a 20 mg/mL injection to 100 mL with water, remove a 10 mL aliquot and add it to 3 mL 0.2% hydrochlorothiazide, make up to 100 mL with water, inject a 20 μL aliquot. Tablets. Grind tablets to a fine powder, weigh out amount equivalent to about 10 mg hydralazine, mix thoroughly with 2 mL 500 mM HCl, make up to 100 mL with water, shake for 2-3 min, filter, discard first 15 mL. 15 mL Filtrate + 1.5 mL 0.2% hydrochlorothiazide, make up to 50 mL with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak phenyl
Mobile phase: MeOH:15 mM KH_2PO_4:glacial acetic acid 0.5:99.4:0.1
Flow rate: 3
Injection volume: 20
Detector: UV 256

CHROMATOGRAM
Retention time: 5
Internal standard: hydrochlorothiazide (8)

OTHER SUBSTANCES
Simultaneous: phenylpropanolamine

KEY WORDS
injections; tablets

REFERENCE
Das Gupta,V. Quantitation of hydralazine hydrochloride in pharmaceutical dosage forms using high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1985**, *8*, 2497–2509.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, sonicate for 10 min with enough 5 mM HCl to give a 30 μg/mL solution, filter, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb CN
Mobile phase: MeOH:buffer 20:80 (Buffer was 7 mM sodium heptanesulfonate:50 mM triethylamine adjusted to pH 3.1 with dilute phosphoric acid 80:20.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: dihydralazine, phthalazine

KEY WORDS
tablets

REFERENCE
Di Pietra,A.M.; Roveri,P.; Gotti,R.; Cavrini,V. Spectrophotometric and chromatographic (HPLC) analysis of hydralazine, dihydralazine and hydrazine after derivatization with 2-nitrocinnamaldehyde, *Farmaco*, **1993**, *48*, 1555–1567.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, add MeCN:5 mM sodium octanesulfonate 15:85, sonicate, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:5 mM sodium octanesulfonate:phosphoric acid 15:85:0.045
Flow rate: 2
Detector: UV 220

CHROMATOGRAM
Retention time: 10.86

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
tablets

REFERENCE
Lessen,T.; Zhao,D.C. Interactions between drug substances and excipients. 1. Fluorescence and HPLC studies of triazolophthalazine derivatives from hydralazine hydrochloride and starch, *J.Pharm.Sci.*, **1996**, *85*, 326–329.

SAMPLE
Matrix: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeOH:buffer 8:92 adjusted to pH 3.0 with phosphoric acid (Buffer was 54 mM NaH_2PO_4 containing 1.24 mM sodium heptanesulfonate.)
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 0 V

CHROMATOGRAM
Retention time: 12.50
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: apomorphine, dopamine, isoproterenol, methoxamine, morphine, norepinephrine, phenylephrine

KEY WORDS
rat; derivatization

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: solutions
Sample preparation: Make up a solution in 40 mM sodium formate and 62 mM formic acid buffer (pH 3.5), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 μm CN (Waters)
Column: 150 × 4.6 5 μm Ultrasphere CN
Mobile phase: MeOH:buffer 15:85 (Buffer was 40 mM sodium formate and 62 mM formic acid, pH 3.5.)
Flow rate: 1
Injection volume: 20
Detector: UV 258

CHROMATOGRAM
Retention time: 3.7
Internal standard: phenylephrine (2.7)

OTHER SUBSTANCES
Simultaneous: phthalazine, degradation products

REFERENCE
Halasi,S.; Nairn,J.G. Quantitative determination of hydralazine hydrochloride and phthalazine in aqueous solutions by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1989**, *12*, 2397–2403.

SAMPLE
Matrix: solutions
Sample preparation: Add a 1 mL aliquot of a solution in MeOH:water 70:30 to 1 mL pH 4.5 acetate buffer, add 50 μL acetic acid, add 1 mL 2.2 mM 2-nitrocinnamaldehyde in EtOH, heat at 70° for 50 min, cool to room temperature, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Hypersil C18
Mobile phase: MeCN:buffer 65:35 (Prepare buffer by adjusting the pH of 50 mM triethylamine to 3.3 with dilute phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 390

CHROMATOGRAM
Retention time: 5.1

OTHER SUBSTANCES
Simultaneous: hydrazine (UV 350)

KEY WORDS
derivatization

REFERENCE
Di Pietra,A.M.; Roveri,P.; Gotti,R.; Cavrini,V. Spectrophotometric and chromatographic (HPLC) analysis of hydralazine, dihydralazine and hydrazine after derivatization with 2-nitrocinnamaldehyde, *Farmaco*, **1993**, *48*, 1555–1567.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 12 μm Dynamax C18 (Rainin)
Mobile phase: MeCN:50 mM acetic acid 20:80
Flow rate: 2

Detector: UV 241

CHROMATOGRAM
Retention time: 1.6

OTHER SUBSTANCES
Simultaneous: metabolites, 3-methyl-s-triazolo[3,4-α]phthalazine

REFERENCE
Hickman,D.; Palamanda,J.R.; Unadkat,J.D.; Sim,E. Enzyme kinetic properties of human recombinant arylamine N-acetyltransferase 2 allotypic variants expressed in *Escherichia coli*, *Biochem.Pharmacol.*, **1995**, *50*, 697–703.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

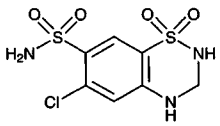
CHROMATOGRAM
Retention time: 6.46 (A), 3.49 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Hydrochlorothiazide



Molecular formula: $C_7H_8ClN_3O_4S_2$
Molecular weight: 297.74
CAS Registry No.: 58-93-5
Merck Index: 4822
Lednicer No.: 1 358

SAMPLE
Matrix: blood
Sample preparation: 500 µL Serum +100 µL 1.25 µg/mL IS + 5 mL MTBE, vortex for 2 min. Centrifuge at 2700 g for 5 min and evaporate the organic phase to dryness under a stream of nitrogen. Dissolve the residue in 200 µL water, add 3 mL toluene, vortex for 2 min, centrifuge at 2700 g for 10 min, discard the toluene layer . Add 3 mL toluene, vortex, centrifuge, discard the toluene layer. Evaporate the aqueous layer to dryness under a stream of nitrogen. Reconstitute the residue in 200 µL mobile phase. Inject a 100 µL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 µm RP-C18
Column: 250 × 4 5 µm LiChrospher 100 RP-C18
Mobile phase: MeCN:THF:200mM pH 7.5 phosphate buffer 5:10:85
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 273

CHROMATOGRAM
Internal standard: hydroflumethiazide
Limit of detection: 3.3 ng/mL
Limit of quantitation: 11.2 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Vervaet,C.; Remon,J.P. Bioavailability of hydrochlorothiazide from pellets, made by extrusion/spheronisation, containing polyethylene glycol 400 as a dissolution enhancer, *Pharm.Res.*, **1997**, *14*, 1644–1646.

SAMPLE
Matrix: blood
Sample preparation: 200 µL Serum + 20 µL 1 M pH 7.0 phosphate buffer, extract with 5 mL MTBE, vortex for 20 s, centrifuge at 2500 g for 10 min. Remove the organic layer and add it to 10 µL 20 µg/mL IS in MeOH, evaporate to dryness at 80° in a vacuum centrifuge, reconstitute the residue with 180 µL mobile phase, inject a 30 µL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 µm endcapped LichroCART RP18
Mobile phase: MeCN:7.5 mM pH 7.3 phosphate buffer 10:90
Column temperature: 40
Flow rate: 0.8
Injection volume: 30
Detector: E, ESA Coulochem II, coulometric cell 5011, first electrode +450 mV, second electrode +630 mV; UV 254